

Cel-Seq primers were used (Hashimshony et al. 2012, PMID: 22939981) to prime RT reaction on mRNA from single cells, containing an Illumina 5' adapter as used in the illumine small RNA kit.

The Illumina 3' adapter from the small RNA kit was then ligated to fragmented amplified RNA, after which the Illumina small RNA kit RT primer was used for cDNA synthesis from the amplified RNA. During PCR, Illumina small RNA kit PCR index primers were used to multiplex libraries into the same sequencing run.

To demultiplex the barcodes after sequencing, read 1 should be parsed in the following manner:

the first 8 basepairs are the Cel-Seq cell barcodes (see list below). The following four basepairs are random basepairs of the unique molecular identifier, which can be used to count individual molecules for each transcript. The rest of read 1 consists of polyT and is not used. Read two is then mapped to the reference genome of choice (hg19 in our case).

1	CATCACGC
2	GTCGTCGC
3	ACGACCGC
4	TGATGCGC
5	CATCAATC
6	GTCGTATC
7	ACGACATC
8	TGATGATC
9	CATCATCC
10	GTCGTTCC
11	ACGACTCC
12	TGATGTCC
13	CATCAGAA
14	GTCGTGAA
15	ACGACGAA
16	TGATGGAA
17	TCACACGC
18	CGTGTGCG
19	GACACCGC
20	ATGTGCGC
21	TCACATCA
22	CGTGTTC
23	GACACTCA
24	ATGTGTCA
25	TCACAGAG
26	CGTGTGAG
27	GACACGAG
28	ATGTGGAG

29	CTAACCGC
30	GCTTGCAG
31	AGCCACGC
32	TAGGTGCG
33	CTAACTCA
34	GCTTGTCA
35	AGCCATCA
36	TAGGTTCA
37	CTAACGAG
38	GCTTGGAG
39	AGCCAGAG
40	TAGGTGAG
41	GAATCCGA
42	ATTGCGGA
43	TCCGACGA
44	CGGATCGA
45	GAATCATC
46	ATTGATTC
47	TCCGAATC
48	CGGATATC
49	GAATCTCG
50	ATTGTCG
51	TCCGATCG
52	CGGATTG
53	GAATCGAT
54	ATTGGGAT
55	TCCGAGAT
56	CGGATGAT
57	AGCGTCGA
58	TAGACCGA
59	CTATGCGA
60	GCTCACGA
61	AGCGTATC
62	TAGACATC
63	CTATGATC
64	GCTCAATC
65	AGCGTTCG
66	TAGACTCG
67	CTATGTCG
68	GCTCATCG
69	AGCGTGAT
70	TAGACGAT
71	CTATGGAT
72	GCTCAGAT

73	ACTGACGA
74	TGCATCGA
75	CAGTCCGA
76	GTACGCGA
77	ACTGAATC
78	TGCATATC
79	CAGTCATC
80	GTACGATC
81	ACTGATCG
82	TGCATTAG
83	CAGTCTCG
84	GTACGTCG
85	ACTGAGAT
86	TGCATGAT
87	CAGTCGAT
88	GTACGGAT
89	TGTACCGA
90	CACTGCGA
91	GTGCACGA
92	ACAGTCGA
93	TGTACATC
94	CACTGATC
95	GTGCAATC
96	ACAGTATC